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Statins enhance cognitive performance in object location test in albino Swiss mice: Involvement of beta-adrenoceptors

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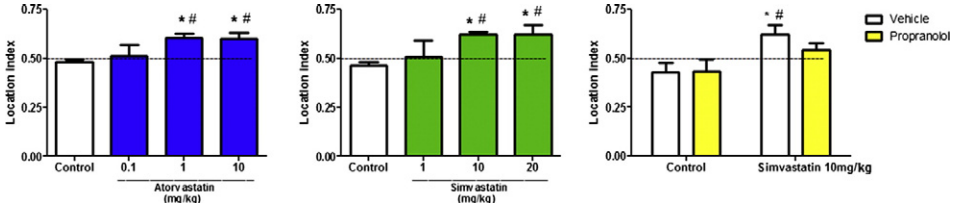
HIGHLIGHTS

- Statins improve performance of Swiss albino mice in the OLT.
- Beta-adrenergic receptor abolishes the beneficial effects of simvastatin.
- Statins do not change the exploratory parameters in the OF.
- Statins do not change the anxiety-like parameters in the EPM.

GRAPHICAL ABSTRACT



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ABSTRACT

Statins are inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, thereby inhibiting cell synthesis of cholesterol and isoprenoids. Moreover, several studies have been evaluating pleiotropic effects of statins, mainly because they present neuroprotective effects in various pathological conditions. However, knowledge about behavioral effects of statins *per se* is relatively scarce. Considering these facts, we aimed to analyze behavioral responses of atorvastatin or simvastatin-treated mice in the open field test, elevated plus maze and object location test. Atorvastatin treatment for 7 consecutive days at 1 mg/kg or 10 mg/kg (v.o.) or simvastatin 10 mg/kg or 20 mg/kg enhanced cognitive performance in object location test when compared to control group (saline-treated mice). Simvastatin effects on mice performance in the object location test was abolished by post-training infusion of the beta-adrenoceptor antagonist propranolol. Atorvastatin and simvastatin did not change the behavioral response in open field and elevated plus-maze (EPM) tests in any of the used doses. These data demonstrate the positive effects of both statins in cognitive processes in mice, without any alteration in locomotor parameters in the open field test or anxiolytic-like behavior in EPM. In conclusion, we demonstrate that atorvastatin and simvastatin *per se* improve the cognitive performance in a rodent model of spatial memory and this effect is related to beta-adrenergic receptors modulation.

1. Introduction

Statins are inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, thereby inhibiting cell synthesis of

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cholesterol and isoprenoids [1]. The HMG-CoA reductase is the pacemaker enzyme of cholesterol synthesis by reducing HMG-CoA to mevalonate [2]. Retrospective studies suggest that the prevalence of Alzheimer Disease (AD) and vascular dementia is lower among patients taking statins, even reducing the levels of Aβ peptides induced by cerebral trauma [3–5]. Additionally, several studies have evaluated pleiotropic effects of statins, mainly because they present neuroprotective effects on pathological conditions [6–8]. Furthermore, the safety of high doses of atorvastatin and simvastatin has been demonstrated in adult humans [9,10].

Several studies in rodent animals are performed to evaluate the neuroprotective effects of statins [11–14]. Statins promote reduction of neurological deficits and increase in synaptogenesis, angiogenesis and neuronal survival in animals exposed to a model of traumatic brain injury [15]. Atorvastatin reduced the seizure activity and the neuronal death in rat hippocampus after seizures induced by kainate [1]. Atorvastatin also reduced the number of convulsing animals and promoted neuroprotection against hippocampal cell death after seizures induced by quinolinic acid, an NMDA receptor agonist [8]. Other members of the statin family, as fluvastatin, simvastatin and pravastatin, have shown differential effects regarding on the intervention schedule against cognitive impairment induced by amyloid-beta (Aβ) peptide infusion, due to a prevention of cholinergic neuronal loss or modulation of glutamatergic system [16,17]. Moreover, clinical data indicates that statin therapy is linked to a reduction in the incidence of depression and anxiety [18], although the mechanisms of action are not yet established.

Despite the evidence of important roles for statins on neurological diseases, the knowledge about behavioral effects of statins *per se* is scarce [19]. It has been demonstrated that treatment with statins prevented spatial memory deficit induced by traumatic brain injury or scopolamine infusion in rodents [15,20,21]. Statin treatment has also been shown to prevent neuronal cell death as well to prevent cognitive deficits induced by Aβ infusion [22,23]. Besides, it has been shown that simvastatin treatment improved the performance of control rats in the object location and passive avoidance tasks [14]. A wide range of mechanisms has been proposed to explain these pleiotropic effects of statins including antioxidant, anti-inflammatory, immunomodulatory, modulation of nitric oxide production and increased expression of brain-derived neurotrophic factor (BDNF) [24–26]. However, the precise molecular mechanisms involved in the memory enhancing effects of the statins remain unknown.

In this study, mice were subjected to three different behavioral tasks. The open field task (OF) represents a new environment and it is used to analyze the locomotor behavior in mice and rats [27]. The elevated plus-maze is the classical approach to evaluate anxiolytic-like behavior in rats and currently is also used in mice [28]. The object location test (OLT) is based on rodents' natural behavior (novelty preference), an innate instinct that drives animals to learn about their environment (discrimination ratio). Additionally, it has been reported that the performance of animals in this task is dependent of the hippocampal function [29].

Considering the necessity for elucidation of the behavioral effects of statins, this study investigated the behavioral effects of atorvastatin and simvastatin treatments in mice submitted to the OF, EPM and OPR tests. Additionally, we evaluated the involvement of beta-adrenergic receptor on the cognitive effects of statin treatment.

2. Materials and methods

2.1. Animals

Male adult Swiss albino mice (3 months old/45 ± 5 g) were kept on a 12-h light/dark cycle (light on at 07.00 a.m.) at a constant temperature of 22 ± 1 °C. They were housed in plastic cages with tap water and commercial food *ad libitum*. All procedures were carried out according to the institutional policies on animal experimental handling, designed to minimize suffering and limit the number of animals used and were approved by the local Ethical Committee for Animal Research. All experiments were performed during the light phase (between 14:00 and 17:00 h) to avoid circadian variations.

2.2. Pharmacological treatments

Total of 203 male Swiss albino mice were employed to study the putative role of statins on behavioral changes, animals were treated orally with atorvastatin (Lipitor Atorvastatin calcium, Pfizer) 0.1, 1 or 10 mg/kg/day, or simvastatin 1, 10 or 20 mg/kg/day once a day during seven consecutive days [7,8]. Control animals were treated with vehicle (NaCl 0.9%) orally for the same period. One day after the last atorvastatin or saline administration animals were submitted to the specifically behavioral task. Object location test, or open field, or elevated plus maze were analyzed in this specific time.

After the initial results, we performed additional experimental procedure to evaluate the involvement of beta-adrenergic receptors in the effects of statins in the OLT (49 animals were employed in these experiments). Mice received 10 mg/kg/day of simvastatin or vehicle (NaCl 0.9%) for seven days. One day after the last simvastatin or vehicle (NaCl 0.9%) animals were submitted to OLT. The animals of two initial groups were treated with the beta-adrenergic receptor antagonist propranolol (2 mg/kg, i.p.; Sigma Chemical Co., St. Louis, U.S.A.) or vehicle (NaCl 0.9%) immediately after the training session. All treatments were done by the administration of 10 μl/g weight of the animal.

2.3. Behavioral tasks

Mice were randomly assigned for treatments. Animals were housed in the communal plastic cages (10 animals per cage). The behavioral task was performed and analyzed by a blinded observer. Every experimental procedure presents animals of each group to comparative analyses. A control experiment procedure was used by two groups to evaluate the effects of propranolol in short term memory of Swiss albino mice in OLT. All experiments were performed during the light phase (between 14:00 and 17:00 h) to avoid circadian variations. In every experiment, the animals are exposed to apparatus in randomized order to minimize the circadian effects in the behavioral analyses.

2.4. Object location test

The OLT was performed in an apparatus consisting of a wooden box chamber (40 cm × 60 cm × 50 cm). Before the experimental sessions, animals (total of 112, distributed in 4 experiments) were habituated to the experimental room for 90 min in dim light conditions. A light bulb was switched on during the experimental sessions. The light intensity was equal in different parts of the apparatus. In the adaptation sessions, mice explored the apparatus for 10 min, with no object. The objects were placed equidistant from two corners, 10 cm apart from the wall. Mice were placed individually into the chamber and performed the task for 10 min. In training sessions, 2 similar objects were utilized. In test sessions, performed 90 min (12 animals, to control the effects of propranolol in short term memory) or 24 h later (total of 100 animals), one object was replaced to the other corner of the chamber. The objects employed were two LEGO® pieces presenting the same texture, size, shape and color. The objects were not known to have any ethological significance for mice [30]. Discrimination ratio was expressed by the ratio TN/(TN + TF), (TN, time spent exploring the novel place; TF, time spent exploring familiar place), both in the training and test sessions. During the inter-trial interval objects were cleaned with 10% ethanol solution to avoid odor cues. Exploration was defined by directing the nose to the object at a distance less than 2 cm and/or touching the object with the nose or forepaws. The time of exploration was measured by two blinded observers, with the use of chronometers.

Animals that explored the objects less than 3 s in a session were excluded from the study [30].

2.5. Open field (OF)

To examine the effect of atorvastatin treatment on spontaneous locomotor activity, the animals (64 mice) were placed for 5 min in the open field arena. The apparatus, a transparent Plexiglas arena (30 × 30 × 15 cm), had a black Plexiglas floor divided by white lines in nine squares (10 cm × 10 cm). The experiments were conducted in a sound-attenuated room and light intensity in the center of the apparatus was 110 lx. The animals were placed in the center of the open field and the movement of each mouse was recorded using a video camera placed above the open field apparatus. The recorded movements were then analyzed using ETHOWATCHER(®) system [31] and the numbers of squares crossed, rearing behavior, distance traveled and velocity performed were calculated.

2.6. Elevated plus-maze test (EPM)

The EPM was made of clear Plexiglas and consisted of two opposed open arms (30 × 5 × 0.25 cm) and two opposed closed arms (30 × 5 × 15 cm), all extending from a central platform (5 × 5 cm), elevated 45 cm from the floor. The apparatus was placed in a small closed room lit by a 15 W red light. The animals (76 mice) were placed in the central platform, facing an enclosed arm, and were observed for a 5 min period. The entries into either arms (open or closed), as well as the time spent in each arm type were recorded (in sec). The ratios "time spent in the open arms/time spent in all (*i.e.* open plus closed) arms" and "frequency of entries into open arms/total entries into all arms" were calculated and multiplied by 100, to yield the percentage of time spent in and of frequency of entries into open arms, respectively. Both parameters are considered to reflect fear-induced inhibition from entering the open arms, and drugs with anxiolytic activity usually increase the time spent in and/or entries into open arms, whereas the reverse holds true for anxiogenic-like drugs. Furthermore, the number of entries into enclosed arms was used as an index of general activity [32].

2.7. Statistical analyses

One-sample t tests were used to determine whether the location index was different from chance performance (50%) in the OLT. For the other behavioral data, comparisons among treatment groups and control were performed by one-way or two-way ANOVA, followed by Newman–Keuls test when appropriate. A value of $p < 0.05$ was considered to be significant in all tests.

3. Results

3.1. Object location test

In the OLT, control group ($t = 1.14$, $p = 0.19$) (0.48 ± 0.03) or atorvastatin 0.1 mg/kg/day treatment ($t = 0.2$, $p = 0.84$) (0.51 ± 0.13) presented location index that did not differ from chance performance, indicating that mice in these groups were not able to identify the spatial alteration in this task (Fig. 1A). However, mice treated with atorvastatin 1 mg/kg/day ($t = 4.11$, $p < 0.01$) ((0.6 ± 0.07) or 10 mg/kg/day ($t = 3.34$; $p < 0.01$) (0.59 ± 0.09) have shown a location index significantly increased from chance performance (Fig. 1A). One-way ANOVA followed by Newman–Keuls post-hoc test indicated that atorvastatin 1 or 10 mg/kg/day treatment is significantly different from control group [$F(3,30) = 4.26$; $p < 0.05$] (Fig. 1A). Location index of mice treated with saline ($t = 1.77$; $p = 0.11$) (0.46 ± 0.06) or simvastatin 1 mg/kg/day ($t = 0.11$; $p = 0.91$) (0.5 ± 0.2) did not differ from chance performance (Fig. 1C). Mice treated with simvastatin 10 (0.62 ± 0.03) or 20 mg/kg/day (0.62 ± 0.11) presented location indexes increased from chance performance, ($t = 2.4$; $p = 0.03$ and $t = 3.1$, $p = 0.02$, respectively) (0.62 ± 0.03; 0.62 ± 0.11) (Fig. 1C). Furthermore, one-way ANOVA followed by Newman–Keuls post-hoc test indicated that simvastatin treatment 10 or 20 mg/kg/day are significantly different from control group [$F(3,26) = 4.31$; $p < 0.05$] (Fig. 1C).

As revealed by two-way ANOVA, all groups presented a similar time spent in both objects in the training and test sessions [atorvastatin: $F(3,30) = 0.32$; $p = 0.8$], [simvastatin: $F(3,26) = 0.17$; $p = 0.91$] and the same decrease in time spent in both objects in the test session [atorvastatin: $F(3,30) = 72.49$; $p < 0.05$] and [simvastatin: $F(3,26) = 32.13$; $p < 0.05$] (Fig. 1B and D). In atorvastatin experiments, time spent in both objects in training and test sessions are, respectively: control group (58.32 ± 25.3; 22.85 ± 9.89), atorvastatin 0.1 mg/kg (50.5 ± 11.48; 19.83 ± 7.27), atorvastatin 1 mg/kg (58.26 ± 30.34; 26.72 ± 14.83) and atorvastatin 10 mg/kg (58.12 ± 21.56; 19.62 ± 5.85). In simvastatin experiments, time spent in both objects in training and test sessions are, respectively: control group (36.36 ± 12.13; 22.66 ± 11.61), simvastatin 1 mg/kg (33.33 ± 5; 23.33 ± 8.98), simvastatin 10 mg/kg (35.38 ± 9.91; 20.87 ± 10.31) and simvastatin 20 mg/kg (39.42 ± 14.08; 22.63 ± 11.36).

A large body of evidence suggests that beta-adrenergic receptor may modulate learning and memory processes evaluated in the passive avoidance, water maze, object recognition and object location tests [33–35]. To evaluate the involvement of beta-adrenergic receptors in the effects of statins in the OLT, mice were treated with the beta-adrenergic receptor antagonist propranolol immediately after the training session. Fig. 2A shows that both vehicle ($t = 2.97$; $p < 0.05$) (0.63 ± 0.1) or propranolol (2 mg/kg) ($t = 3.21$; $p < 0.05$) (0.61 ± 0.08) treated mice were able to discriminate the spatial alteration when tested 90 min after the training session. This indicates that propranolol (2 mg/kg) did not interfere with consolidation of short-term memory in the OLT.

As indicated in Fig. 2B, treatment with propranolol immediately after training session prevented the increase in the location index induced by simvastatin in the OLT. One-sample t-test revealed that simvastatin group presented location index higher than chance performance ($t = 2.39$; $p < 0.05$) (0.62 ± 0.14), while control group ($t = 1.43$; $p = 0.18$) (0.43 ± 0.15), propranolol group ($t = 1.05$; $p = 0.32$) (0.43 ± 0.17) and propranolol + simvastatin group ($t = 1.54$; $p = 0.28$) (0.54 ± 0.1) presented location indexes with no difference from chance performance. The two-way ANOVA revealed significant difference for simvastatin treatment [$F(1,30) = 11.68$; $p < 0.01$], but no difference for propranolol treatment [$F(1,30) = 1.21$; $p = 0.26$] and simvastatin–propranolol interaction [$F(1,30) = 1.47$; $p = 0.23$] (Fig. 2B).

3.2. Open-field

The administration of atorvastatin (0.1–10 mg/kg) or simvastatin (1–20 mg/kg) did not affect mice exploration in the open field test (number of crossings after atorvastatin treatment: $F(3,28) = 2.33$, $p = 0.09$; (72.13 ± 15.99; 83.13 ± 20.51; 94 ± 15.51; control group: 74.88 ± 20.15); number of rearings after atorvastatin treatment: $F(3,28) = 2.04$, $p = 0.13$; (25.25 ± 6.36; 25.25 ± 9.67; 32.75 ± 6.08, control group: 24.88 ± 7.53); number of crossings after simvastatin treatment: $F(3,28) = 1.91$, $p = 0.33$ (71.13 ± 20.39; 85.56 ± 11.71; 77.71 ± 13.38; control group 79.63 ± 16.66); number of rearings after simvastatin treatment: $F(3,28) = 1.67$, $p = 0.19$; (26.38 ± 11.38; 32.89 ± 5.37; 28.71 ± 4.42; control group: 33.38 ± 6.36) (Fig. 3).

3.3. Elevated plus-maze test (EPM)

Fig. 4A–C shows that treatment with atorvastatin (0.1–10 mg/kg) did not alter the percentage of time spent in the open arms [$F(3,31) = 0.47$; $p = 0.7$] (12.77 ± 8.85; 11.12 ± 9.53; 16.37 ± 9.88;

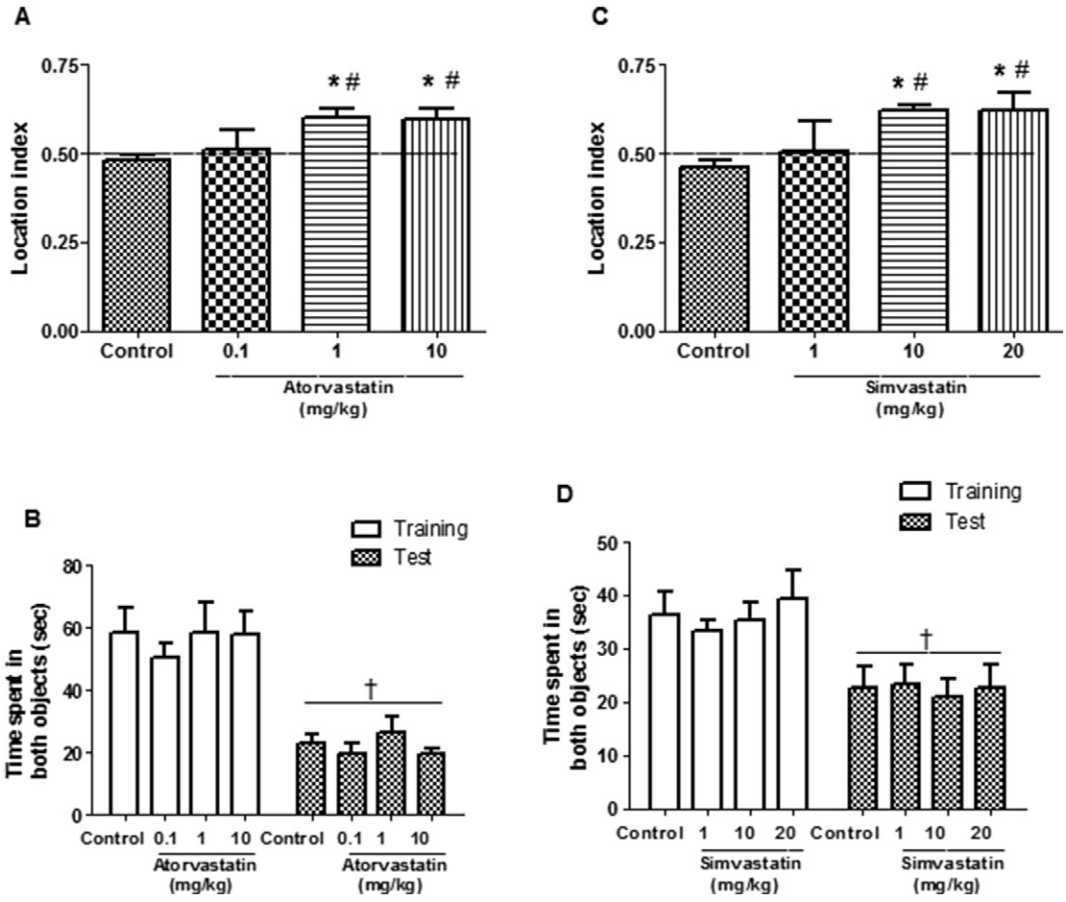

Fig. 1. Effects of statin treatment on mice performance in the object location test. The animals were treated with vehicle (saline 0.9%, v.o.), atorvastatin (0.1–10.0 mg/kg/day, v.o.) or simvastatin (1–20 mg/kg/day, v.o.) for seven days. Behavioral analysis was performed 24 h after the last day of statin treatment. Mice were allowed to explore two identical objects for 10 min on the acquisition trial. The test trial was conducted 24 h after the acquisition trial. A: Effect of atorvastatin treatment on object location in mice. B: Time spent exploring both objects during training and test sessions after atorvastatin treatment. C: Effect of simvastatin treatment on object location in mice. D: Time spent exploring both objects during training and test sessions after simvastatin treatment. Results are presented as means ± S.E.M. N = 6–10 per group. *p < 0.05 *versus* chance level (% 50 of displaced object investigation in test trial). #p < 0.05 *versus* control group. †p < 0.05 *versus* training session. ANOVA followed by Newman–Keuls post hoc test.

control group: 16.46 ± 14.78), percentage of entries in the open arms [F(3,31) = 0.12; p = 0.94] (25.22 ± 16.66; 23.22 ± 10.53; 25.45 ± 7.37; control group: 27.45 ± 19.24) or total arms entries [F(3,31) = 2.04; p = 0.12] (12.75 ± 2.12; 12.88 ± 3.44; 17.38 ± 5.15; control group: 14 ± 5.15) in the EPM. Similarly, simvastatin treatment (1–20 mg/kg) did not promote any alteration the percentage of time spent in the open arms (7.31 ± 5.13; 11.33 ± 8.45; 11.67 ± 9.22; control group: 12.52 ± 9.21) [F(3,37) = 0.59; p = 0.62], percentage of entries in the open arms (19.5 ± 9.6; 23.76 ± 13.15; 22.3 ± 14.9; control group: 26.97 ± 11.19) [F(3,37) = 0.54; p = 0.65] or total arms entries (12.43 ± 2.07; 13.17 ± 4.34; 13.36 ± 4.38; control group: 14 ± 5.15) [F(3,37) = 0.19; p = 0.89] in the EPM (Fig. 4D–F).

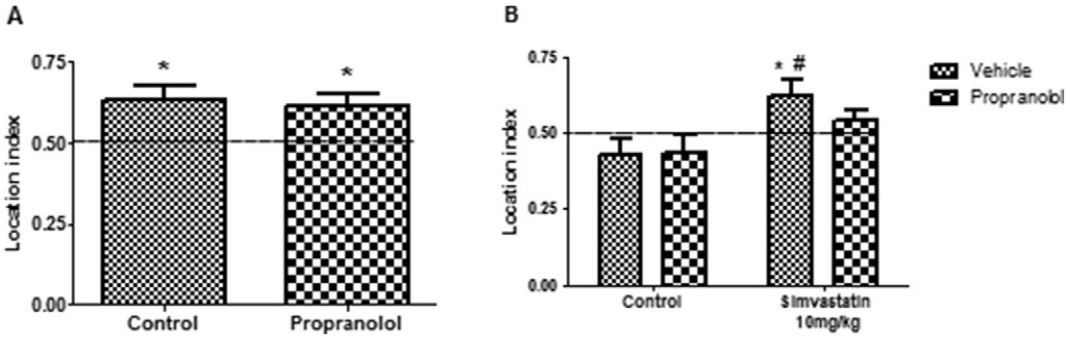

Fig. 2. Effects of propranolol treatment on the cognitive effect of simvastatin in the object location test in mice. The animals were treated with vehicle (saline 0.9%, v.o.) or simvastatin (10 mg/kg/day, v.o.) for seven days. Behavioral analysis was performed 24 h after the last day of statin treatment. Mice were allowed to explore two identical objects for 10 min on the acquisition trial. Immediately after the training session mice were injected with propranolol (2 mg/kg, i.p.). The test trial was conducted 90 min or 24 h after the acquisition trial. A: Effect of propranolol treatment on object location test in mice with 90 min intertrial interval. B: Effect of propranolol treatment on the simvastatin induced cognitive effect in the object location test in mice. Results are presented as means ± S.E.M. N = 6–9 per group. *p < 0.05 *versus* chance level (% 50 of displaced object investigation in test trial). #p < 0.05 *versus* control group. Two-way ANOVA followed by Newman–Keuls post hoc test.

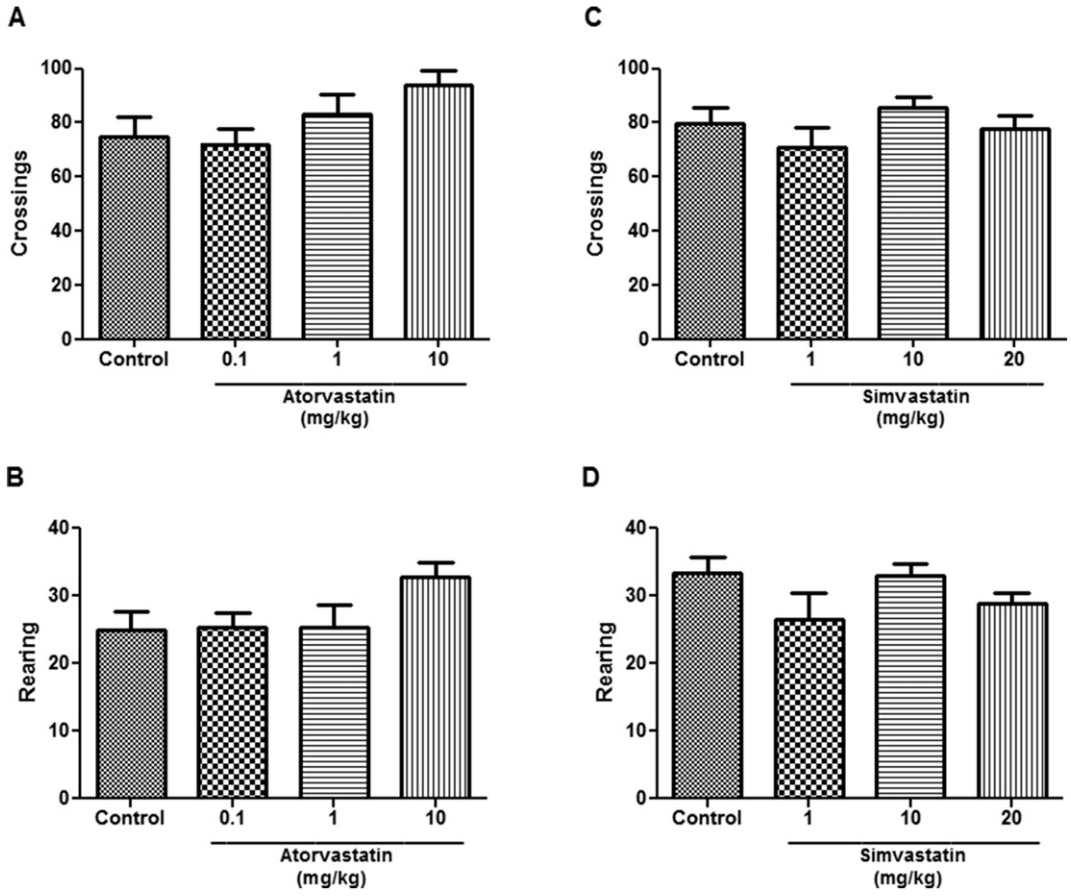

Fig. 3. Effects of statin treatment in the open field test. The animals were treated with vehicle (saline 0.9%, v.o.), atorvastatin (0.1–10.0 mg/kg/day, v.o.) or simvastatin (1–20 mg/kg/day, v.o.) for seven days. Behavioral analysis was performed 24 h after the last day of statin treatment. A: Number of crossings in the open field after atorvastatin treatment. B: Number of rearings in the open field after atorvastatin treatment. C: Number of crossings in the open field after simvastatin treatment. D: Number of rearings in the open field after simvastatin treatment. N = 8–9 per group. One-way ANOVA.

4. Discussion

In the present study, we demonstrated that mice treated with atorvastatin or simvastatin was able to discriminate the spatial alteration in the OLT for a long period than vehicle-treated mice. The effect of simvastatin treatment on mice performance in the OLT was abolished by beta-adrenergic receptor blockade with propranolol, suggesting that at least in part this mnemonic effect involves the beta-adrenergic system. Besides, we demonstrated that treatment with atorvastatin or simvastatin did not promote any change in individual performance in exploratory- and anxiety-like tasks in Swiss mice. These findings demonstrated that short treatment of atorvastatin and simvastatin *per se* promoted an improvement in cognitive response, besides the cellular protective effects that has been previously demonstrated in animal models of neurological disorders.

It has been shown that atorvastatin is able to promote improvement in the consolidation and retrieval phases of memory [21]. Moreover, atorvastatin (5 mg/kg) is also able to protect the deficit in the spatial recognition performance in mice treated with scopolamine [21]. We demonstrated that treatment with atorvastatin (1 or 10 mg/kg) and simvastatin (10 or 20 mg/kg) for 7 days, promoted a cognition improvement of mice in the OLT, however, low doses of atorvastatin (0.1 mg/kg) and simvastatin (1 mg/kg) did not improve the cognition performance.

Simvastatin has been shown to improve the performance of Sprague–Dawley rats in object location and in inhibitory avoidance tests [14]. Additionally, simvastatin enhanced LTP in the hippocampus, a region involved in the learning/memory process in these tasks [36]. However, it is important to mention that prolonged treatment with statins may occasionally promote adverse effects such as liver toxicity and myopathy [37,38]. Despite different periods of treatment, both atorvastatin and simvastatin have been shown to promote a decrease in degeneration of hippocampal neurons and to improvement in short-term and long-term functional performances after traumatic brain injury in mice [39].

Both statin treatments did not elicit any changes in open field and elevated plus-maze exploration. In accordance to this, Ludka et al. demonstrated that a single administration of atorvastatin in a range dose of 0.01 to 30 mg/kg, did not cause any changes in locomotor parameters [40]. It has been shown that 1 week of atorvastatin treatment (10 and 20 mg/kg) improved the deficit promoted by stroke in the locomotors patterns, but atorvastatin *per se* did not cause any changes in OF [41]. The literature demonstrates that 2 weeks of simvastatin treatment do not change exploratory patterns in open field in rats, however 4 weeks of simvastatin (10 mg/kg) treatment promotes an increase in distance traveled in the open field task [14,19]. Moreover, 4 weeks of simvastatin treatment increased the time spent exploring open arms in EPM, reflecting a reduction in anxiety-like behavior [19]. Additionally, it has been demonstrated that 8 weeks of simvastatin treatment improves motor coordination on the rotarod, while slowing down motor speed on the horizontal bar of the coat-hanger [42]. In fact, the behavioral responses to statin treatment depend on time of treatment and on the statin employed. Therefore, 7 days of treatment of atorvastatin or simvastatin did not alter the locomotor parameters here evaluated.

Atorvastatin and simvastatin induce neurogenesis and improve spatial learning after traumatic brain injury [43]. In addition, atorvastatin is

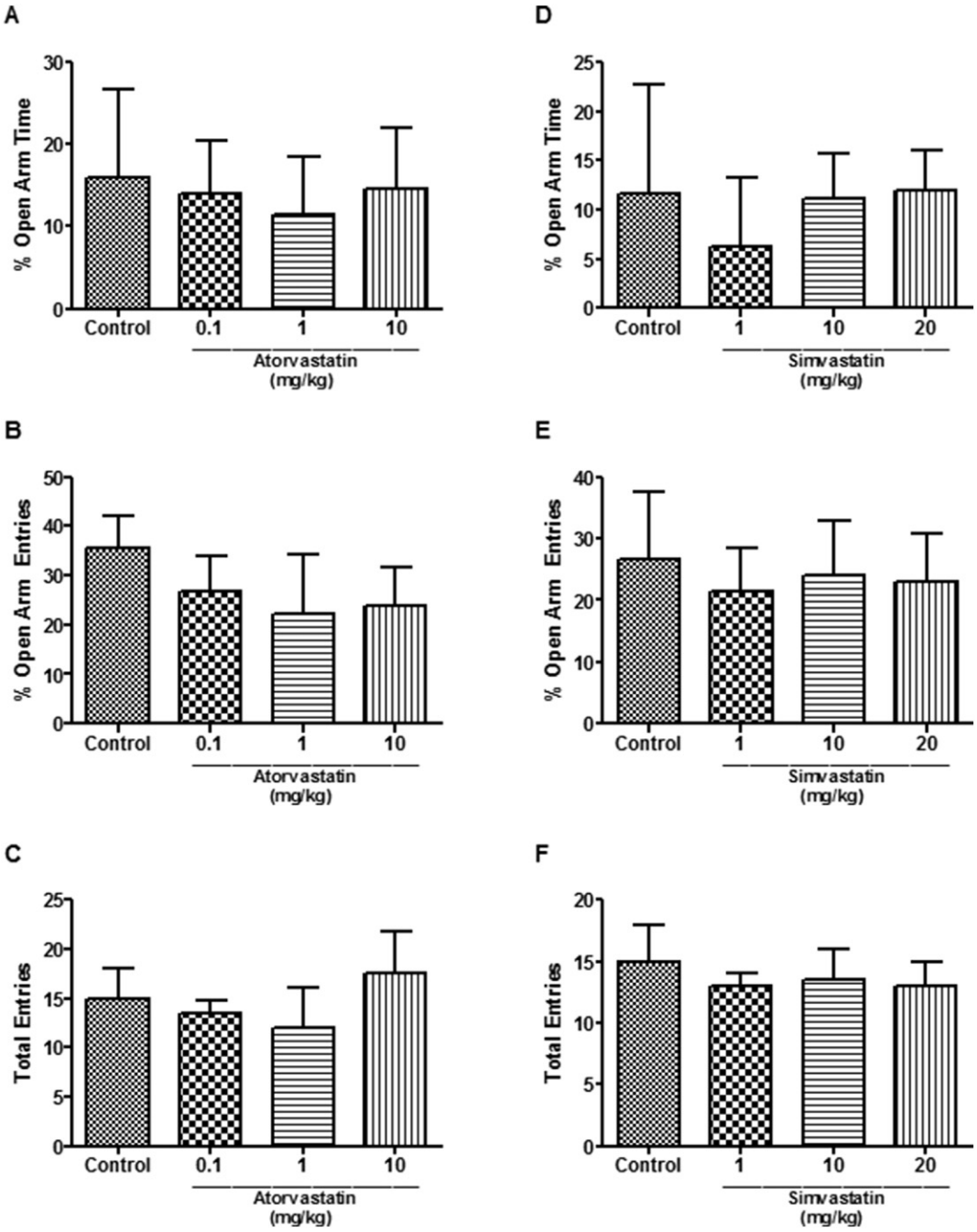

Fig. 4. Effects of atorvastatin treatment in the elevated plus-maze test. The animals were treated with vehicle (saline 0.9%, v.o.), atorvastatin (0.1–10.0 mg/kg/day, v.o.) or simvastatin (1–20 mg/kg/day, v.o.) for seven days. Behavioral analysis was performed 24 h after the last day of statin treatment. A: Percentage of time spent in the open arms (% open arm time) after atorvastatin treatment. B: Percentage of number of entries into open arms (% open arms entry) after atorvastatin treatment. C: Total arms entries after atorvastatin treatment (total entries). D: Percentage of time spent in the open arms after simvastatin treatment. E: Percentage of number of entries into open arms after simvastatin treatment. F: Total arms entries after simvastatin treatment. Values expressed are as mean ± S.E.M. One-way ANOVA.

able to promote an improvement in consolidation and retrieval phases of memory in a Y maze task, although nitric oxide (NO) is involved in atorvastatin effect in the consolidation but not in retrieval phase of memory [21]. Ludka et al. demonstrated that an acute treatment with atorvastatin in low doses improved BDNF protein levels in the hippocampus of Swiss albino mice [40]. BDNF has multiple effects on sustaining and evoking elements of brain plasticity and it is involved in the mnemonic process [44]. Therefore, statins present cognitive effects on animals and, at least in part, these effects might be related to a modulation of BDNF expression and nitric oxide synthesis, although the exact mechanisms by which statins promote cognitive improvement need to be further explored.

It has been proposed that the activation of β-adrenergic receptors is an important link between NO synthesis and expression of genes involved in the learning process, such as BDNF [45]. In this way, since NO signaling pathway and BDNF expression have been related to the beneficial effects of statins, we aimed to evaluate if statin effect could be abolished by noradrenergic beta-receptor blockade. Our findings demonstrated that propranolol administered immediately after training disrupted the effect of simvastatin in the location memory test. It is

known that β-adrenergic receptor blockade impairs the consolidation of memory training experiences both in aversive tasks [46] and tasks involving low levels of emotional arousal, such as novel object recognition [47,48]. Moreover, it has been demonstrated that the noradrenergic system of basolateral amygdala modulates long-term memory consolidation of object-in-context recognition training [49]. Besides the effects on beta-adrenergic system, statin treatment has also been shown to modulate alpha noradrenergic receptors. Kandasamy et al. demonstrated that atorvastatin treatment prevented hyporreactivity to noradrenalin in the aorta from septic mice in part by increasing α(1D)-adrenoceptor mRNA expression [50]. In fact, other G-protein coupled receptors have been implicated in the pleiotropic effects of statins. Atorvastatin antidepressant-like effect in the tail suspension test has been shown to be dependent on 5-HT1A/2A/C receptor modulation [51]. However, the exact mechanism through which statins modulate G-protein coupled receptors, such as noradrenergic and serotonergic receptors remains unclear.

Nevertheless, few articles demonstrate the direct interaction between statin treatment and beta-adrenergic receptors in the central nervous system. In cardiac tissue, Clements and Jamali [52] demonstrated that 4 days of pravastatin 6 mg/kg (twice day) improve the potency of propranolol to prolong PR interval, the authors suggest that the interaction effects of statin and propranolol occur at the pharmacodynamic level because pravastatin did not influence the pharmacokinetics of propranolol. Additionally, pravastatin 6 mg/kg (twice a day) did not change the binding of propranolol to plasma proteins (Clements and Jamali, 2007). It may be possible that statin treatment modulates beta-adrenergic receptors directly through the modulation of isoprenylation of Gγ subunits and/or indirectly through modulation of intracellular signaling pathways [53]. In fact, statins appear to reverse the down-regulatory effect of inflammation on β-adrenergic receptors [54]. Therefore, one interesting point to be elucidated is the direct and/or indirect interactions between statins and β-adrenergic receptor activity.

In conclusion, atorvastatin or simvastatin treatment for 7 days presented beneficial effects on learning and memory in mice in the object location test. Furthermore, both statin treatments for 7 days do not change the exploratory behavior in open field and elevated plus-maze, suggesting no collateral effects that could impair a short treatment with atorvastatin and simvastatin. Additionally, the effect of simvastatin on mice performance in the OLT involves, at least partially, the beta-adrenergic system. However, more studies are necessary to elucidate the mechanisms through which statins modulate the mnemonic events.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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